LMT/VJT/bmw
June 16, 2003

PATENT APPLICATION
DOCKET NO. 2825.1013-002
Expedited Procedure under 37 C.F.R. 1.116
Examining Group 1636

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Hiten D. Madhani and Eric S. Lander		
Application No.:	09/439,969	Group:	1636
Filed:	November 12, 1999	Examiner:	Gerald G. Leffers, Jr.
Confirmation No.:	2363		

For: TARGETS OF THE MAP KINASE PATHWAY IN THE DEVELOPMENTAL SWITCH IN YEAST

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450

on June 16, 2003 (Date) Beverly Weinberger (Signature)

Beverly Weinberger
Typed or printed name of person signing certificate

MAIL STOP AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Transmitted herewith is an Amendment After Final Rejection for filing in the above-identified application.

[ ] Small entity status of this application under 37 C.F.R. 1.9 and 1.27 has been established by a Small Entity Statement previously submitted.

[ ] A Small Entity Statement to establish small entity status under 37 C.F.R. 1.9 and 1.27 is enclosed.

The fee has been calculated as shown below:

	(COL. 1) CLAIMS REMAINING AFTER AMENDMENT		(COL. 2) HIGHEST NO. PREVIOUSLY PAID FOR	(COL. 3) PRESENT EXTRA
TOTAL	15	MINUS	* 20	0
INDEP	7	MINUS	** 7	0
☐ FIRST PRESENTATION OF MULTIPLE DEP. CLAIM				

* not fewer than 20
** not fewer than 3

SMALL ENTITY			OR	OTHER THAN SMALL ENTITY	
	RATE	ADDIT. FEE		RATE	ADDIT. FEE
X	$9	$		X $18	$
X	$42	$		X $84	$
+	$140	$		+ $280	$
	TOTAL =	$ 0		TOTAL =	$ 0

Please charge Deposit Account No. 08-0380 for the following fees:

[ ]	Petition for [ ] month Extension of Time	$
[ ]	Amendment Fee	$
[ ]	Other Fees:	
		$
		$
	TOTAL:	$ 0

A check is enclosed in payment of the following fees:

[X]	Petition for two months Extension of Time	$ 410
[ ]	Amendment Fee	$
[X]	Other Fees:	
	Notice of Appeal	$ 320
		$
	TOTAL:	$ 730

[X] A general authorization is hereby granted to charge Deposit Account No. 08-0380 for any fees required under 37 C.F.R. 1.16 and 1.17 in order to maintain pendency of this application. A copy of this authorization is enclosed for accounting purposes.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Lisa M. Treannie

Lisa M. Treannie
Registration No.: 41,368
Telephone (978) 341-0036
Facsimile (978) 341-0136

Concord, Massachusetts 01742-9133

Dated: 6/16/03

@PFDesktop\::ODMA/MHODMA/iManage;391435;1
LMT/VJT/bmw
June 16, 2003

PATENT APPLICATION
Attorney's Docket No.: 2825.1013-002
Expedited Procedure under 37 C.F.R. § 1.116
Examining Group 1636

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Hiten D. Madhani and Eric S. Lander		
Application No.:	09/439,969	Group:	1636
Filed:	November 12, 1999	Examiner:	Gerald G. Leffers, Jr.
Confirmation No.:	2363		
For:	TARGETS OF THE MAP KINASE PATHWAY IN THE DEVELOPMENTAL SWITCH IN YEAST		

#20/C NE
Yuta
6/21/03

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450

on June 16, 2003 (Date) Beverly Weinberger (Signature)

Beverly Weinberger
Typed or printed name of person signing certificate

AMENDMENT AFTER FINAL REJECTION UNDER 37 C.F.R. § 1.116

Do Not Enter /AP 7-8-03

MAIL STOP AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This Amendment After Final Rejection is being filed in response to the Final Office Action mailed from the U.S. Patent and Trademark Office on January 14, 2003, in the above-identified application.

An extension of time and a Notice of Appeal from the Final Office Action dated January 14, 2003, with appropriate fees, are being filed concurrently.

Please amend the application as follows:

In the Specification

Please delete the paragraph at page 4, lines 26 through 27.

Please delete the paragraph at page 5, lines 2 through 3.

Please replace the paragraph at page 3, line 28 through page 4, line 8 with the following paragraph:

Compounds or molecules which activate or inhibit PGUI can also be identified. For example, activators of this pectinase can be identified by expressing PGUI in an appropriate host cell (e.g., a bacterial or yeast cell), contacting the cells with (e.g., by culturing them in the presence of) candidate activators (compounds or molecules to be assessed for their effects on PGUI activity) and determining their effect on PGUI (e.g., whether they enhance or activate PGUI expression or activity, repress or decrease PGUI expression or activity or have no effect). Compounds which enhance or activate PGUI expression or activity are activators; those which repress or decrease its expression or activity are inhibitors). Activators and inhibitors of PGUI are also the subject of this invention.

Please replace the paragraph at page 4, lines 9 through 24 with the following paragraph:

Also the subject of this invention is a method of inhibiting (totally or partially) invasion of a host, particularly a plant host by a fungus (i.e., a method of inhibiting fungal invasion of a host). In the method, a compound or molecule which inhibits the MAPK pathway or specifically inhibits TOT10/YELO33W is applied to a host (e.g., by application to a plant surface) in such a manner that it contacts the fungus (e.g., the yeast) and inhibits one or more components of the MAPK pathway, such as TOT10/YELO33W. For example, an inhibitor can be a compound which binds and inhibits TOT10/YELO33W; galacturonic acid; or a mimic of galacturonic acid which represses TOT10/YELO33W. In a specific embodiment, the method of inhibiting fungal invasion of a host comprises contacting a fungus (e.g., a yeast) with a compound which inhibits he MAPK pathway and/or inhibits TOT10/YELO33W, in sufficient quantity that inhibition of the MAPK pathway and/or inhibition of TOT10/YELO33W occurs, thereby inhibiting fungal

invasion of the host. In a further embodiment, the host is a plant and the compound is applied to a plant surface (e.g., root, leaf, stem) or seed in such a manner that it contacts the fungus and inhibits (totally or partially) the ability of the fungus to invade.

Please replace the paragraph at page 4 line 28 with the following paragraph:

Figure 1 lists MAPK pathway targets.

Please replace the paragraph at page 5, line 1 with the following paragraph:

Figure 2 summarizes results of systematic knockout experiments.

Please replace the paragraph at page 5, lines 4 through 5 with the following paragraph:

Figure 3 shows genes selectively induced by the plant-specific carbohydrate polygalacturonic acid and its hydrolysis product.

Please replace the paragraph at page 5, lines 6 through 7 with the following paragraph:

Figure 4 shows genes selectively repressed by the plant-specific carbohydrate polygalacturonic acid and its hydrolysis product.

Please replace the paragraph at page 5, line 8 with the following paragraph:

Figures 5A-C is a compilation of MAPK data, sorted as TEC1-high copy/tec1Δ.

Please replace the paragraph at page 5, lines 9 through 10 with the following paragraph:

Figure 6 shows results of profiling experiments with polygalacturonic acid (PGA) and galacturonic acid (GA), sorted by PGA/YPD.

Please replace the paragraph at page 5, lines 11 through 12 with the following paragraph:

Figure 7 shows results of profiling experiments with polygalacturonic acid (PGA) and galacturonic acid (GA), sorted by GA/YPD.

Please replace the paragraph at page 5, lines 13 through 14 with the following paragraph:

Figure 8 shows a flow chart of homologous genes induced by the filamentation and mating MAPK pathways.

Please replace the paragraph at page 5, lines 15 through 16 with the following paragraph:

Figures 9A-B shows a listing of genes whose expression is reduced in STE12$^-$, STE7$^-$ but show greater than double an effect with Tec1.

Please replace the paragraph at page 5, line 19 through page 6, line 2 with the following paragraph:

Described herein is work carried out to identify and study the targets of the MAP kinase pathway in order to understand how signaling cascades control a developmental switch in this *Saccharomyces cerevisiae* model system. The pathway consists of four kinases Ste20 (PAK), Ste11 (MEKK), Ste7 (MEK) and Kss1 (MAPK), which display both positive and negative control over the pathway, as well as a heterodimeric transcription factor Tec1-STE12. STE7, STE11 and STE20 also participate in the yeast mating MAPK pathway. Global expression patterns in haploid cells under rich medium conditions were examined in the following mutants: wild type tec1Δ Ste12Δ, Ste7Δ, TEC1-overexpression, and STE11-4 (an activated mutant of the MEKK). Expression profiling was carried out using nucleic acid arrays (chips) such as described in WO95/11995. One chip set was used per sample (chips with obvious defects were redone).

Please replace the paragraph at page 6, lines 3 through 11 with the following paragraph:

18 genes were identified that show strong regulation by the pathway-specific transcription factor Tec1 (i.e. 3.5-20X difference in expression comparing TEC1-overexpression to tec1Δ). These 18 genes are as follows:

PGU1 (YJR153W)

FLO11 (YIR019C)

ORF (YEL033W)

SRD1 (YCR018C)

ORF (YKR105C)

ORF (YOR225W)

FLO5 (YHR211W)

DDR48 (YMR173W)

ORF (YLR042C)

ORF (YER158C)

ORF (YIL117C)

ORF (YHL049C; _f)

ORF (YLR434C)

ORF (YBR113W)

ORF (YIR013C)

PHO84 (YML123C)

KTR2 (YKR061W)

SJH1 (YIL002C)

Almost all of these also show a consistent dependency on STE7, STE7, and STE12. In tec1Δ, ste12Δ and ste7Δ ("down" mutants), expression of most of these genes were down-regulated by 1 to 1.5, 1.5 to 2, 2 to 2.5, 2.5 to 3 and greater than 3 fold. In contrast, in STE11-4 and TEC HC ("up" mutants), must of these genes were up-regulated by 1 to 1.5, 1.5 to 2, 2 to 2.5, 2.5 to 3, 3 to 3.5 and greater than 3.5 fold. One gene that was known previously to be regulated by the pathway, FLO11 (which encodes a cell surface protein required for pseudohyphal growth) is the second-most strongly regulated target. Detailed studies were performed on one of these targets, PGU1, which encodes a secreted carbohydrate-destroying enzyme. This enzyme breaks down a key component of plant cell walls, polygalacturonic acid (which is the main component of pectin).

Please replace the paragraph at page 6, lines 12 through 18 with the following paragraph:

Remarkably, galacturonic acid, the breakdown product of pectin, causes the strong repression of a gene, TOT10/YEL033W, which is turned on in the filamentation MAPK pathway and which these results have shown is required for invasion and filamentation. Thus, work described herein has identified a new regulatory circuit in yeast in which a signal from the host feeds back on the filamentation/invasion pathway. This is the first demonstration of a specific interaction between yeast and its plant host. Figures 1-9 show the data in detail.

Please replace the paragraph at page 7, lines 6 through 16 with the following paragraph:

Accordingly, the invention relates to a method of inhibiting invasion of a host by a fungus, comprising contacting the fungus with a compound which inhibits expression of a gene expressed in the filamentation MAPK pathway that enhances the filamentation MAPK pathway, in sufficient quantity that inhibition of the expression of the gene occurs, thereby inhibiting invasion of the host by the fungus. In one embodiment, the host is a plant, and the compound is applied to a plant surface (e.g., a leaf, a root, a stem, a flower) in such a manner that it contacts the fungus. An effective amount of the compound can be determined empirically by assessing expression levels of the gene to be inhibited. In a preferred embodiment, the gene is TOT10/YELO33W. In one embodiment, the fungus is a yeast, such as *Saccharomyces cerevisiae*.

Please replace the paragraph at page 9, lines 13 through 22 with the following paragraph:

The invention also relates to a method of identifying an agent which inhibits the filamentation MAPK pathway in a fungus, comprising the steps of providing an expression vector comprising a nucleic acid molecule of a gene which is expressed in the filamentation MAPK pathway; transforming a suitable host cell with said expression vector under conditions suitable for expression of said gene; contacting said host cell with an agent to be tested; and comparing the expression of said gene in the presence of the agent with the expression of said gene in the absence of said agent, wherein if the expression of said gene is lower in the presence of the agent than in the absence of the agent, then the agent is an inhibitor of the filamentation MAPK pathway in a fungus. In one embodiment, the gene is TOT10/YELO33W.

Please replace the paragraph at page 10, lines 17 through 21 with the following paragraph:

The invention further includes a method of inhibiting invasion of a host by a fungus, comprising contacting the fungus with a compound which enhances expression of a gene expressed in the filamentation MAPK pathway that inhibits the pathway, in sufficient quantity that enhancement of the expression of the gene occurs, thereby inhibiting invasion of the host by the fungus.